

WHAT IS CLAIMED IS:

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1. A method for changing acoustic reflectivity of an ultrasound target, the method comprising (1) administering to the target, a nongaseous acoustic imaging substance which binds to the target and produces a change in acoustic reflectivity with a change in temperature and (2) changing the temperature to produce a measurable change in acoustic reflectivity of the nongaseous acoustic imaging substance bound to the target.
- 10 2. The method according to claim 1 wherein the nongaseous acoustic imaging substance comprises an emulsion which contains a liquid fluorocarbon.
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132/ 3. The method according to claim 5 wherein the fluorocarbon is perfluorooctane.
- 15 4. The method according to claim 1 wherein the nongaseous acoustic imaging substance comprises a ligand which binds to the target.
- ✓ 20 5. The method according to claim 4 wherein the ligand is an antibody, a fragment of an antibody, a polypeptide, a peptidomimetic, a polysaccharide, an aptamer, a lipid, a nucleic acid or a lectin.
- 13 6. The method according to claim 5 wherein the nongaseous acoustic imaging substance comprises a ligand conjugated with a biotin agent, an emulsion conjugated with a biotin agent and an avidan agent immobilized to the biotin-conjugated ligand and to the biotin-conjugated emulsion.
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7. The method according to claim 5 wherein the nongaseous imaging substance comprises a ligand immobilized to a primer substance which is immobilized to an emulsion.

5 8. The method according to claim 1 wherein the emulsion further comprises a biologically active agent.

10 ✓ 9. The method according to claim 8 wherein the biologically active agent is an antineoplastic agent, a radiopharmaceutical, a hormone, an analgesic agent, a nonsteroidal anti-inflammatory agent, an anesthetic agent, a sedative, a neuromuscular blocker, an antimicrobial agent, an antiparasitic agent, an antiviral agent, an interferon, a nitrate, an acne preparation, an androgenic agent, an antidiabetic agent, an anti-gout agent, an antihistamine, an antitussive agent, a decongestant, an expectorant, an antiulcer agent, a laxative, an anticoagulant, an immunization agent, 15 an anticonvulsant agent, an anti-parkinsonian agent, an estrogenic agent, a thyroid agent, or an iron-containing anti-anemia agent.

20 ✓ 10. The method according to claim 1 wherein the nongaseous acoustic imaging substance further comprises a magnetic resonance imaging substance, an electron spin resonance imaging substance, a spectroscopic imaging substance, a positron emission tomography imaging substance, an optical imaging substance, an x-ray imaging substance, a nuclear medicine imaging substance or a combination thereof.

25 ✓ 11 The method according to claim 1 wherein the spectroscopic imaging substance comprises a nuclear magnetic resonance spectroscopic imaging substance or a raman spectroscopy imaging substance.

✓ 12. The method according to claim 11 wherein the nongaseous imaging agent comprises a paramagnetic or superparamagnetic element, a radioactive nuclide, or a photoactive agent.

04/5 sub 13. The method according to claim 1 wherein changing the temperature comprises energizing the bound substance to increase temperature of the bound substance and enhance acoustic reflectivity of the surface.

10 ✓ 14. The method according to claim 13 wherein the energizing is performed by generating energy from ultrasound, shortwave, microwave, magnetic radiation, electromagnetic energy or a combination thereof.

15. The method according to claim 1 wherein changing the temperature of the bound substance comprises reducing the temperature of the bound substance to produce a measurable decrease in acoustic reflectivity of the target.

16. The method according to 15 wherein reducing the temperature of the bound substance is performed as part of cryotherapy or heart bypass surgery.

20 17. The method according to claim 1 wherein changing the temperature comprises changing the temperature of the bound substance by at least 5°C.

25 SUB A8 18. A method for measuring enhanced acoustic reflectivity of an ultrasound target, the method comprising (1) administering to the target, a nongaseous acoustic imaging substance which binds to the target and produces a change in acoustic reflectivity with a change in temperature and (2) changing the temperature to produce a measurable change in acoustic reflectivity of the nongaseous acoustic imaging substance bound to the target, and (3) detecting change in acoustic reflectivity of the bound substance at increased

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19. The method according to claim 18 wherein detecting comprises (a) measuring reflectivity prior to changing the temperature of the bound substance; (b) measuring reflectivity after changing the temperature of the bound substance; and (c) determining the change in reflectivity after changing the temperature of the bound substance compared to reflectivity prior to changing the temperature of the bound substance.

20. The method according to claim 19 wherein the nongaseous acoustic imaging substance comprises an emulsion which contains a liquid fluorocarbon.

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21. The method according to claim 20 wherein the fluorocarbon is perfluorooctane.

22. The method according to claim 19 wherein the nongaseous acoustic imaging substance comprises a ligand which binds to the target.

23. The method according to claim 22 wherein the ligand is an antibody, a fragment of an antibody, a polypeptide, a peptidomimetic, a polysaccharide, an aptamer, a lipid, a nucleic acid or a lectin.

24. The method according to claim 23 wherein the nongaseous acoustic imaging substance comprises a ligand conjugated with a biotin agent, an emulsion conjugated with a biotin agent and an avidin agent immobilized to the biotin-conjugated ligand and to the biotin-conjugated emulsion.

139 <sup>sub</sup> 25. The method according to claim 23 wherein the nongaseous imaging substance comprises a ligand immobilized to a primer substance which is immobilized to an emulsion.

5 26. The method according to claim 19 wherein the emulsion further comprises a biologically active agent.

10 27. The method according to claim 26 wherein the biologically active agent is an antineoplastic agent, a radiopharmaceutical, a hormone, an analgesic agent, a nonsteroidal anti-inflammatory agent, an anesthetic agent, a sedative, a neuromuscular blocker, an antimicrobial agent, an antiparasitic agent, an antiviral agent, an interferon, a nitrate, an acne preparation, an androgenic agent, an antidiabetic agent, an anti-gout agent, an antihistamine, an antitussive agent, a decongestant, an expectorant, an antiulcer agent, a laxative, an anticoagulant, an  
15 immunization agent, an anticonvulsant agent, an anti-parkinsonian agent, an estrogenic agent, a thyroid agent, or an iron-containing anti-anemia agent.

20 28. The method according to claim 19 wherein the nongaseous acoustic imaging substance further comprises a magnetic resonance imaging substance, an electron spin resonance imaging substance, a spectroscopic imaging substance, a positron emission tomography imaging substance, an optical imaging substance, an x-ray imaging substance, a nuclear medicine imaging substance or a combination thereof.

25 29. The method according to claim 19 wherein the spectroscopic imaging substance comprises a nuclear magnetic resonance spectroscopic imaging substance or a raman spectroscopy imaging substance.

30. The method according to claim 29 wherein the nongaseous imaging agent comprises a paramagnetic or superparamagnetic element, a radioactive nuclide, or a photoactive agent.

31. The method according to claim 19 wherein changing the temperature comprises energizing the bound substance to increase temperature of the bound substance and enhance acoustic reflectivity of the surface.

32. The method according to claim 31 wherein the energizing is performed by generating energy from ultrasound, shortwave, microwave, magnetic radiation, electromagnetic energy or a combination thereof.

33. The method according to claim 19 wherein changing the temperature of the bound substance comprises reducing the temperature of the bound substance to produce a measurable decrease in acoustic reflectivity of the target.

34. The method according to 33 wherein reducing the temperature of the bound substance is performed as part of cryotherapy or heart bypass surgery.

35. The method according to claim 19 wherein changing the temperature comprises changing the temperature of the bound substance by at least 5°C.

36. A method for monitoring temperature of a tissue in a patient, the method comprising (1) administering to the patient, a nongaseous acoustic imaging substance which binds to the tissue and changes acoustic reflectivity with changes in temperature, (2) detecting acoustic reflectivity of the nongaseous acoustic imaging substance bound to the tissue (3) calculating temperature of the nongaseous acoustic imaging substance bound to the tissue.

37. The method according to claim 36 wherein the method monitors a change in temperature, wherein the method further comprises changing the temperature of the tissue and the nongaseous acoustic imaging substance bound to the tissue, and wherein detecting comprises detecting the change in acoustic reflectivity of the nongaseous acoustic imaging substance bound to the tissue.

38. The method according to claim 36 wherein the nongaseous acoustic imaging substance comprises an emulsion which contains a liquid fluorocarbon.

39. The method according to claim 38 wherein the fluorocarbon is perfluorooctane.

40. The method according to claim 36 wherein the nongaseous acoustic imaging substance comprises a ligand which binds to the tissue.

41. The method according to claim 40 wherein the ligand is an antibody, a fragment of an antibody, a polypeptide, a peptidomimetic, a polysaccharide, an aptamer, a lipid, a nucleic acid or a lectin.

42. The method according to claim 41 wherein the nongaseous acoustic imaging substance comprises a ligand conjugated with a biotin agent, an emulsion conjugated with a biotin agent and an avidin agent immobilized to the biotin-conjugated ligand and to the biotin-conjugated emulsion.

43. The method according to claim 41 wherein the nongaseous imaging substance comprises a ligand immobilized to a primer substance which is immobilized to an emulsion.

44. The method according to claim 36 wherein the emulsion further comprises a biologically active agent.

5           45. The method according to claim 44 wherein the biologically active agent is an antineoplastic agent, a radiopharmaceutical, a hormone, an analgesic agent, a nonsteroidal anti-inflammatory agent, an anesthetic agent, a sedative, a neuromuscular blocker, an antimicrobial agent, an antiparasitic agent, an antiviral agent, an interferon, a nitrate, an acne preparation, an androgenic agent, an  
10       antidiabetic agent, an anti-gout agent, an antihistamine, an antitussive agent, a decongestant, an expectorant, an antiulcer agent, a laxative, an anticoagulant, an immunization agent, an anticonvulsant agent, an anti-parkinsonian agent, an estrogenic agent, a thyroid agent, or an iron-containing anti-anemia agent.

15           46. The method according to claim 36 wherein the nongaseous acoustic imaging substance further comprises a magnetic resonance imaging substance, an electron spin resonance imaging substance, a spectroscopic imaging substance, a positron emission tomography imaging substance, an optical imaging substance, an x-ray imaging substance, a nuclear medicine imaging substance or a combination  
20       thereof.

          47. The method according to claim 46 wherein the spectroscopic imaging substance comprises a nuclear magnetic resonance spectroscopic imaging substance or a raman spectroscopy imaging substance.

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          48. The method according to claim 47 wherein the nongaseous acoustic imaging agent comprises a paramagnetic or superparamagnetic element, a radioactive nuclide, or a photoactive agent.



49. The method according to claim 36 wherein changing the temperature comprises energizing the bound substance to increase temperature of the bound substance and enhance acoustic reflectivity of the surface.

5 50. The method according to claim 49 wherein the energizing is performed by generating energy from ultrasound, shortwave, microwave, magnetic radiation, electromagnetic energy or a combination thereof.

10 51. The method according to claim 36 wherein changing the temperature of the bound substance comprises reducing the temperature of the bound substance to produce a measurable decrease in acoustic reflectivity of the target.

15 52. The method according to 51 wherein reducing the temperature of the bound substance is performed as part of cryotherapy or heart bypass surgery.

53. The method according to claim 36 wherein changing the temperature comprises changing the temperature of the bound substance by at least 5°C.

20 54. A device for measuring changes in temperature of a target having a temperature-sensitive acoustic imaging substance bound thereto, the device comprising a component configured to change the temperature of the acoustic imaging substance, an ultrasound source configured to transmit acoustic energy to the target, an ultrasound detecting component configured to measure acoustic reflectivity of the surface and a comparator which determines acoustic reflectivity of the target upon  
25 changing temperature relative to acoustic reflectivity of the target in absence of changing temperature.

55. The device according to claim 54 wherein the comparator determines difference in acoustic reflectivity of the target prior to and after changing temperature of the acoustic imaging substance bound to the target.

5 56. The device according to claim 55 wherein the comparator determines the difference in acoustic reflectivity of the target upon changing temperature of the acoustic imaging substance bound to the target, compared to acoustic reflectivity of the target after the changed temperature of the acoustic imaging substance bound to the target is diminished.

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57. The device according to claim 54 wherein the ultrasound source and the ultrasound imaging component comprise at least one ultrasonic transducer.

15 58. The device according to claim 54 wherein the component configured to change the temperature of the acoustic imaging substance comprises an energy source.

59. The device according to claim 58 wherein the energy source produces ultrasound, shortwave, microwave, magnetic radiation, electromagnetic energy or a combination thereof.

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60. The device according to claim 59 wherein the energy source is an ultrasound energy source having an intensity of greater than  $0.1 \text{ W/cm}^2$  and less than  $2000 \text{ W/cm}^2$ .

25 61. The device according to claim 54 wherein the component configured to change the temperature of the acoustic imaging substance is an energy absorber.

62. The device according to claim 61 wherein the energy absorber comprises a cryogenic probe.

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A9 63. The device according to claim 54 wherein the ~~component~~ configured to change the temperature of the acoustic imaging substance is configured to change the temperature of the ~~bound~~ substance by at least 5°C.

10 64. The device according to claim 54 wherein the comparator is configured to produce an image comprising the difference in acoustic reflectivity of the surface prior to and after increasing temperature of the acoustic imaging substance.

15 65. The device according to claim 64 wherein the comparator is configured to produce a colorized image comprising the difference in acoustic reflectivity of the surface prior to and after increasing temperature of the acoustic imaging substance.

20 66. The device according to claim 54 further comprising a component configured to perform magnetic resonance imaging, electron spin resonance imaging, spectroscopic imaging, positron emission tomography imaging, optical imaging, x-ray imaging nuclear medicine imaging or a combination thereof.

67. The method according to claim 66 wherein the spectroscopic imaging comprises nuclear magnetic resonance spectroscopic imaging or a raman spectroscopy imaging.

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